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MHC Class II Molecules and Immunoglobulins on Peripheral Blood Lymphocytes of the Bottlenosed Dolphin, *Tursiops truncatus*

TRACY A. ROMANO, SAM H. RIDGWAY, AND VITO QUARANTA
Department of Neurobiology and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642 (T.A.R.); Naval Ocean Systems Center, San Diego, California 92152 (T.A.R., S.H.R.); Department of Immunology IMM8, Research Institute of Scripps Clinic, La Jolla, California 92037 (V.Q.)

ABSTRACT The immune system of marine mammals is of comparative interest because of its adaptation to the aquatic environment. Little information, however, is available on its cellular and molecular components. Here, we used a cross-reactive antibody to MHC class II molecules and an immunoglobulin-specific antiserum for identifying these molecular species on lymphocytes of the bottlenosed dolphin, *Tursiops truncatus*. Limited structural analyses indicated that class II molecules and immunoglobulins of dolphin closely resemble those of other vertebrates. In the peripheral blood of most land mammals both class II and immunoglobulins are usually found on B but not T lymphocytes. Expression of immunoglobulins on dolphin peripheral blood lymphocytes suggests a ratio of B cells to T cells comparable to that of land mammals. However, unlike the majority of land mammals, virtually 100% of the peripheral T cells display pronounced expression of class II molecules, generally considered an indication of T cell activation. It is therefore possible that the physiology of T cell activation has unusual attributes in the dolphin. It is especially interesting that some land mammals, namely swine (ungulates) and dogs and cats (carnivores), also express class II molecules on peripheral blood T lymphocytes. Since ungulates and carnivores are thought to share a common distant ancestry with toothed whales, the evolutionary history may be more relevant than the environmental history in determining these unusual attributes.

Many aspects of the immune system of terrestrial mammals are well understood, particularly in man and mouse. Conversely, very little is known about the immune system of marine mammals, especially the totally aquatic cetaceans. This group, which includes dolphins, porpoises and whales, is of special interest because they first developed as terrestrial mammals, then left the land some 55 to 60 million years ago for life in the water (Gingerich et al., '83). The cetaceans are thought to have evolved from primitive insectivores and possibly to have shared common ancestors with ungulates and carnivores (Slijper, '62). The evolutionary background brings about the question of what modifications have occurred in their immune system during the adaptations of these mammals to life in the ocean.

The cetacean which has been most readily available for biomedical studies is the bottlenosed dolphin, *Tursiops truncatus*. The hematology of this cetacean species has been well studied (Medway and Geraci, '64; Ridgway et al., '70). The animals show a strong leukocytic response to infection. Normal healthy dolphins have white blood cell counts of about 10,000/mm^3 of which approximately 20% are peripheral blood lymphocytes (PBL).

A basic question to ask is whether dolphins, having lived in an aquatic environment for millions of years, possess the basic components of the immune system, namely T and B lymphocytes, and if so, how the proportions of T and B lymphocytes in dolphins compare with terrestrial mammals.

There are only two studies on the classification of PBL of dolphins. Mumford et al. ('75) found that dolphin (*Tursiops truncatus*) PBL were stimulated more by pokeweed (a human B lymphocyte mitogen) than phytohemagglutinin (a T lymphocyte mitogen). On the other hand, Colgrove ('78) found that concanavalin A (a T cell mitogen) produced a greater response than either phytohemagglutinin or pokeweed. A third, more indirect study showed that dolphin sera contained more agglutinins for human B lymphocytes than for human T lymphocytes (Hohn et al., '83). No cell surface markers, however,

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Address reprint requests to Tracy Romano, Department of Neurobiology & Anatomy, University of Rochester School of Medicine & Dentistry, Box 603, 601 Elmwood Avenue, Rochester, NY 14642.

were used in these studies. Therefore, no positive identification of dolphin lymphocyte subsets was possible and, as Cavagnolo ('79) pointed out, no attempts have been made to measure the T and B cell ratio in marine mammals. Such data are instead available for many terrestrial mammals, including cow (Emery et al., '87; Lewin et al., '85), pig (Lewin et al., '85; McCauley and Hartmann, '82; Pescovitz et al., '84; Saalmuller and Reddehase, '88), horse (Lewin et al., '85), sheep (Lewin et al., '85), dog (Turnwald et al., '88), and cat (Kuramochi et al., '87; Taylor, '75).

In this study, we set out to identify lymphocyte subsets in the bottlenosed dolphin (*Tursiops truncatus*). To this end, we used an antiserum to dolphin immunoglobulins which we produced and characterized, and a monoclonal antibody to human MHC class II molecules which we show cross-reacts with the dolphin class II homologues.

MATERIALS AND METHODS

Antibodies

The monoclonal antibody Q5/13, to non-polymorphic epitopes of human MHC class II molecules HLA-DR, -DP, and -DQ, has been described (Quaranta et al., '80). The monoclonal antibodies Q2/70, Q2/80, Q5/6, and their antigenic determinants on human class II molecules have been described (Quaranta et al., '81). The monoclonal antibody W6/32, to non-polymorphic epitopes of human class I molecules (Parham et al., '79), was a gift from P. Parham (Stanford University, Palo Alto, CA). Antisera to dolphin immunoglobulins (DIg) were generated as follows. DIg were purified from dolphin serum by precipitation with 50% saturated ammonium sulfate, followed by ion exchange chromatography on DEAE cellulose (Andrew and Titus, '90a). Fab2 fragments were obtained by pepsin digestion according to the protocol of Andrew and Titus ('90b). Antisera were raised in rabbits by subcutaneous injection of 0.3 mg of Fab2 in complete Freund's adjuvant (CFA), followed by 1 mg in CFA every two weeks thereafter. After the third injection, rabbits were bled every other week. Antisera were purified by affinity chromatography on purified DIg bound to Sepharose. Fab2 or Fab fragments from these purified antibodies or from monoclonal antibodies were obtained by pepsin digestion at pH 4.0, followed by dialysis against PBS. Double-immunodiffusion was carried out as described by Horbeck ('90).

Dolphin blood samples

Blood samples of 20 ml each were obtained from subjects (both male and female) of *Tursiops truncatus* on the ventral aspect of the tail stock. During the studies the dolphins were clinically healthy and total white blood cell and total lymphocyte counts remained within normal ranges (Medway and Geraci, '64; Ridgway et al., '70). The blood samples were mixed with EDTA to prevent coagulation.

Lymphocyte isolation

Eight milliliters of blood were carefully placed on 4 ml of Ficoll-Paque (Pharmacia) in 15 ml tubes and centrifuged for 30 min at 1,500 rpm at room temperature. The recovered mononuclear cell layer was washed twice with RPMI 1640 supplemented with 10% fetal bovine serum, 200 mM L-glutamine, pen-step, 1M Hepes buffer, and 2 mM EDTA, and then incubated with 0.17 M ammonium chloride for lysing of red blood cells. The lymphocytes were counted on a hemocytometer, with trypan blue exclusion as a measure of viability (< 90% in all experiments shown). The cells were then washed twice in Hank's balanced salt solution (HBSS) and resuspended to a final concentration of 0.5×10^6 cells/ml.

Lymphocyte radiolabeling and immunoprecipitations

For radioactive labeling, lymphocytes were cultured at 10^7/ml overnight in methionine-free culture media, containing 1 mCi/ml ^{35}S-methionine. After washing, cells were lysed in non-ionic detergent and immunoprecipitated as described (Quaranta et al., '84). Eluted samples were analyzed by SDS-PAGE (Laemmli, '70) and two-dimensional gel electrophoresis (NEPHGE and SDS-PAGE) (O'Farrell et al., '77) followed by fluorography (Bonner and Laskey, '74).

Indirect immunofluorescence and FACS analyses

The lymphocyte suspension was aliquoted in Eppendorf tubes and centrifuged in an Eppendorf centrifuge at 10,000 rpm for 10 sec. The supernatant was aspirated and the cells were incubated with 50 μl of antibody at a concentration of 10 μg/ml for 1 h. The cells were washed 3× with HBSS before either rabbit Ig-specific goat Fab2 conjugated to fluorescein isothiocyanate (FITC), or mouse Ig-specific goat Fab2 conjugated to phycoerythrin (PE) secondary antibodies was added. After 1 h incubation, samples were washed twice in PBS and fixed in 500 μl of 1% paraformaldehyde. For double labeling, samples were sequentially stained first with one antibody and the appropriate second label, and then the other antibody. Samples were analyzed on a Fluorescent Activated Cell Sorter (FACS IV, Becton Dickinson). Forward/side scatter plots were obtained

for each subject. In some cases, stained cells were inspected with a Zeiss microscope, equipped with epifluorescence. When observed in light microscopy, the majority of cells in the Ficoll preparation presented a typical lymphocyte morphology (i.e., approximately 10–15 μm in diameter, rounded with low cytoplasm). Moreover, when collecting FACS data, lymphocytes from each subject were gated based on their size and low degree of granularity. The remaining cells probably represented monocytes and contaminating granulocytes.

Mitogen stimulation

Isolated dolphin PBL were cultured with the mitogens, concanavalin A (ConA), and phytohemagglutinin (PHA) for 48 h. The cells were then pulsed with 50 μl of tritiated thymidine (1 μCi/ml) and harvested 18 h later. The uptake of tritiated thymidine was measured on a beta counter and expressed as counts per minute (cpm).

RESULTS

A monoclonal antibody to monomorphic determinants of human MHC class II molecules, Q5/13, was tested in immunoprecipitation vs. detergent lysates of radiolabeled dolphin PBL. SDS-PAGE readily visualized two reactive bands, with relative masses closely resembling that of the α (M_r = 34,000) and β (M_r = 29,000) chains of human class II molecules (Fig. 1). Under non-reducing conditions, the M_r = 29,000 band migrated faster, a typical behavior of class II β chains. These results indicated that monoclonal Q5/13 binds to a cross-reactive epitope on dolphin class II molecules.

In order to investigate the distribution of the Q5/13 epitope on dolphin lymphocyte populations, PBL from three subjects (Tt490M, Tt675F, and Tt043F), 1 male and 2 females, were analyzed with

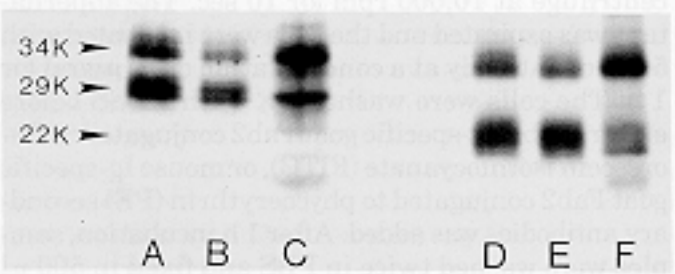

Fig. 1. SDS-PAGE profiles of molecules immunoprecipitated with class II–specific antibody Q5/13 from radiolabeled detergent lysates of PBL from Tt455 (lanes **A,D**) and Tt0 (lanes **B,E**). For comparison, immunoprecipitates with the same antibody and a human B lymphoid cell line, LG-2, are shown (lanes **C,F**). A–C, reducing conditions; D–F, non-reducing conditions. Relevant part of autoradiogram is shown.

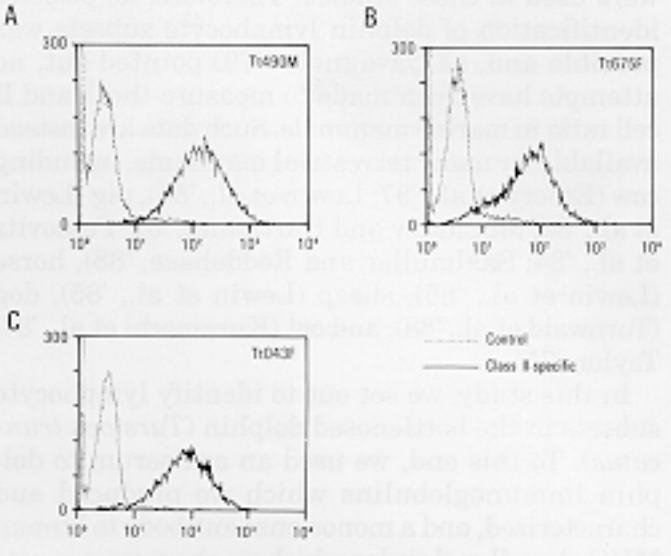

Fig. 2. FACS analyses of PBL from indicated subjects, reacted with either control antibody W6/32 Fab2, vs. human class I molecules, or with class II–specific monoclonal Q5/13 Fab2. y-axis, cell number; x-axis, fluorescence intensity (log scale).

Q5/13 by flow cytometry. Fab2 fragments from this antibody were used to avoid binding to Fc receptors. More than 98% of gated lymphocytes from subject Tt490M were strongly positive, compared to lymphocytes labeled with monoclonal W6/32 as a negative control (Fig. 2). Tt675F and Tt043F had approximately 90% and 95%, respectively, of their gated lymphocytes shifted to positive fluorescence from control. Although there were differences in the degree of fluorescence among the three subjects (Fig. 2), these remained consistent on an individual basis from experiment to experiment. On occasion, stained lymphocytes from Tt043F resolved in a biphasic distribution, with the dull-staining cells amounting to about 40% (not shown).

Reactivity of Q5/13 with three unrelated subjects suggested that the corresponding class II epitope recognized by this antibody could be monomorphic in dolphin, as it is in human. This point was further investigated by serological and biochemical means.

PBL from a total of 18 apparently unrelated subjects were tested by indirect immunofluorescence, and were all positive with Q5/13 (not shown). In every case, 100% of the PBL were stained, in agreement with the flow cytometry results. In these same experiments, three additional monoclonal antibodies to human class II molecules were tested, Q2/70, Q2/80, and Q5/6. Each of these antibodies was positive with most subjects (not shown), but only about 50–80% of lymphocytes were stained, perhaps due to lower affinity of binding compared to Q5/13. A

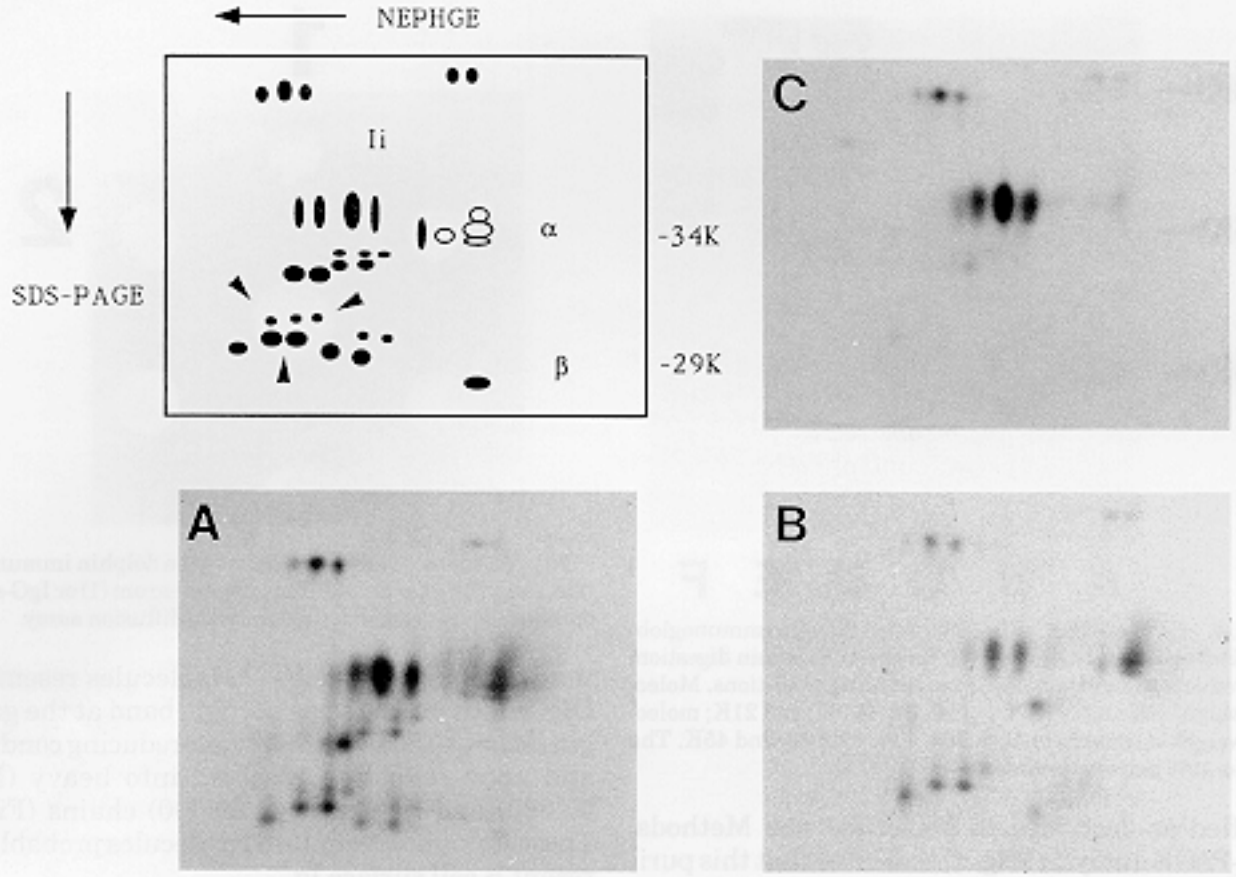

Fig. 3. Two-dimensional gel electrophoresis (NEPHGE and SDS-PAGE) of detergent lysates from dolphin PBL, intrinsically labeled with [^{35}S]methionine and immunoprecipitated with mAb Q5/13, to the β chain of class II molecules (A,B) or antiserum 351, to the invariant chain of class II molecules (C). Samples from two subjects Tg419M (A,C) and Tt043F (B) were analyzed. The diagram of A depicts the correspondence between spots and class II subunits. Note the divergence of the β chain spots between the two subjects. The single common group of β spots is bordered by arrowheads.

monoclonal antibody to human class I molecules, W6/32, was completely negative.

The cross-reactivity of several monoclonal antibodies with the PBL of many unrelated dolphin subjects raised the possibility that MHC class II molecules may not be polymorphic in dolphin. We tested this possibility biochemically, by two-dimensional (two-D) gel electrophoresis of dolphin class II molecules immunoprecipitated by Q5/13. Two subjects were investigated. Dolphin class II molecules display a two-D pattern similar to that of human class II (Fig. 3). The position of the invariant chain (a non-polymorphic, intracellular subunit of class II molecules) (Nowell and Quaranta, '85) spots was positively identified by immunoprecipitation with the anti-invariant chain antiserum 351. The relative position of α and β subunits is indicated in the figure, by analogy to man and mouse. In both samples, the position of the α chain spots, and that of one group of β chain spots, is similar. The important finding, however, is that 11 additional spots in the Tg419M sample, present in the β chain region, are not present in the Tt043F sample. Conversely, 4–5 spots in the Tt043F sample, probably β chains, are not found in the Tg419M sample. These differences are too large to be explained by metabolic labeling differences, and strongly suggest that in dolphin the β chain of class II molecules is encoded by polymorphic alleles.

The finding that essentially 100% of peripheral blood lymphocytes in dolphin are positive for class II molecules deserved further investigation, since in most mammals class II molecules are expressed on B but not T lymphocytes (i.e., only about 15% of PBL). To clarify this point, first we had to generate an antiserum capable of recognizing specifically dolphin immunoglobulins on B lymphocytes. To this end, dolphin immunoglobulins (DIg) were

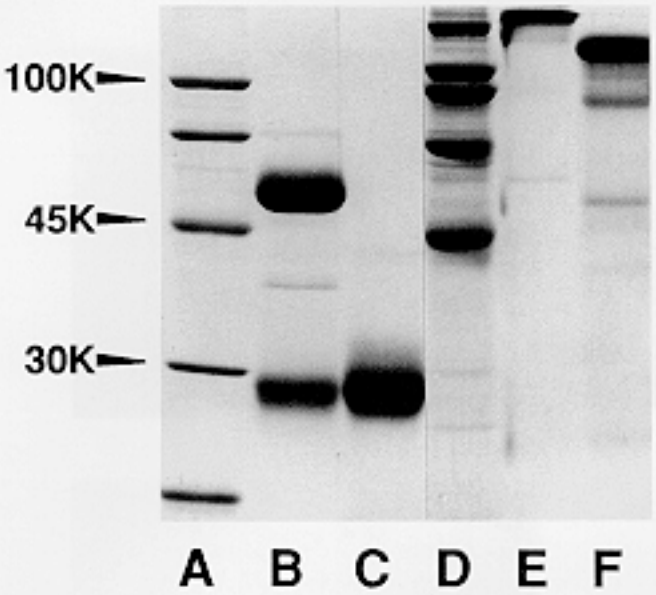

Fig. 4. SDS-PAGE profiles of purified dolphin immunoglobulins before (**lanes B,E**) and after (**lanes C,F**) pepsin digestion. B,C, reducing conditions: E,F, non-reducing conditions. Molecular weight standards in A = 100, 66, 45, 30, and 21K; molecular weight standards in D = 200, 116, 100, 66, and 45K. The gel was 10% polyacrylamide.

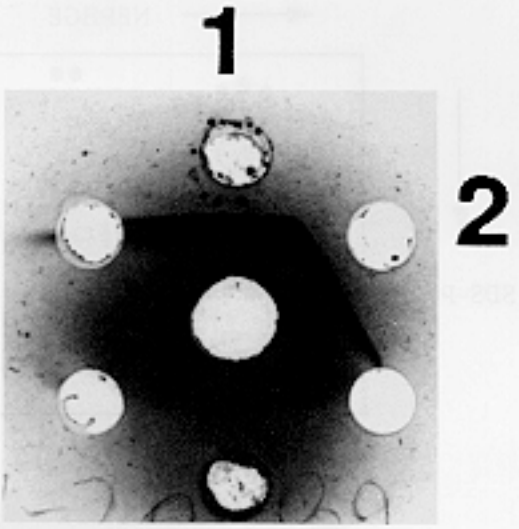

Fig. 5. Identity lines formed between dolphin immunoglobulin antiserum (center well) and dolphin serum (1) or IgG-specific dolphin serum (2) in a double-immunodiffusion assay.

purified as described in Materials and Methods. SDS-PAGE analysis (Fig. 4) indicated that this purified material resolved under reducing conditions as two bands, with relative masses of 55,000 and 25,000 Daltons. These sizes are consistent with the reported mass of heavy and light chains, respectively, of immunoglobulins. Under non-reducing conditions, the two bands migrated as a single band at approximately 150,000 Daltons, suggesting that they are disulfide bridged, another property of immunoglobulin heavy and light chains. The purified material was then treated with pepsin under conditions previously shown to cause cleavage of immunoglobulin molecules into Fab2 fragments. SDS-PAGE analysis showed that the pepsin digested material migrated at M_r = 30,000 under reducing and M_r = 120,000 under non-reducing conditions, consistent with size and disulfide bridging of Fab2 fragments (Fig. 4). Based on these results, we conclude that the material purified represents a dolphin immunoglobulin fraction, probably of the IgG class.

Fab2 fragments were injected into rabbits, as described in Materials and Methods. In double-immunodiffusion experiments, the resulting antisera reacted with the purified IgG preparation as well as dolphin serum causing an identity line (Fig. 5). Furthermore, immunoprecipitation of radiolabeled dolphin PBL detergent lysates indicated that these antisera reacted with molecules resembling DIg, which migrated as a single band at the gel origin (M_r = >150,000) under non-reducing conditions and upon reduction resolved into heavy (M_r = 55,000) and light (M_r = 25,000) chains (Fig. 6). These immunoprecipitated molecules probably represent B cell surface Ig.

Taken together, these results indicated that we had produced antisera specific for dolphin immunoglobulins, which could be used to identify surface Ig-bearing B lymphocytes in the peripheral blood of dolphins.

To this end, DIg-specific antibodies were purified from these antisera by affinity chromatography over immobilized DIg, and to eliminate the possibility of binding to Fc receptors, Fab2 fragments were made from these purified preparations.

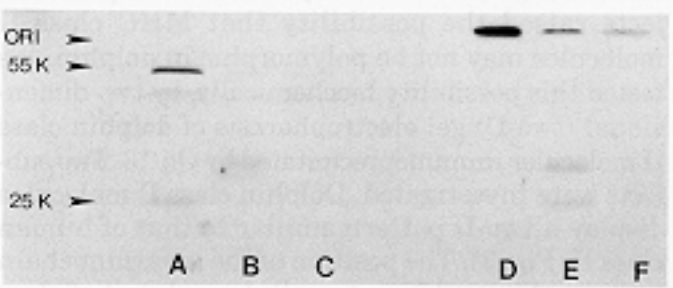

Fig. 6. SDS-PAGE profiles of molecules immunoprecipitated with DIg-specific antiserum (**lanes A,D**) or class II–specific antibody Q5/13 (**lanes B,E**) or control normal rabbit serum (**lanes C,F**) from radiolabeled detergent lysates of PBL from subject Tt0. A–C, reducing conditions; D–F, non-reducing conditions. Relevant part of gel is shown. ORI, origin of the gel. The gel was 12.5% acrylamide, to resolve the light chains.

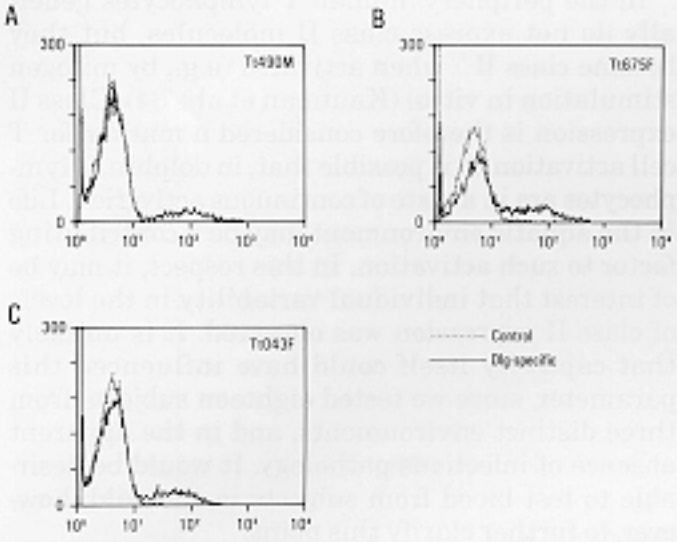

Fig. 7. FACS analyses of PBL from indicated subjects, reacted with either control Fab2 from normal rabbit IgG, or with DIg-specific antibodies Fab2. Vertical axis = cell number; horizontal axis = fluorescence intensity (log scale).

FACS staining with the DIg-specific Fab2 (Fig. 7) showed a positive PBL population between 10 and 15% in all three subjects tested, compared to control normal rabbit Ig Fab2. These results indicate that in the dolphin, like many other mammals, a low percentage of PBL are B lymphocytes and therefore suggests that the remaining dolphin class II–positive cells are probably T lymphocytes.

This was further investigated by two-color fluorescence. Dolphin PBL were first stained with DIg-specific Fab2 and FITC-conjugated rabbit Ig-specific Fab2, followed by Q5/13 and PE-conjugated mouse Ig-specific Fab2. Contour plots from a representative experiment (cells from Tt490M) are shown in Figure 8. A small population of lymphocytes is both DIg and class II positive, while a much larger population is class II positive, DIg negative. The percentages are in close approximation (7% DIg^+, class II^+; 93% DIg^-, class II^+) to the single histogram statistics obtained for the single labeling with DIg-specific antibody and Q5/13. Furthermore, in all experiments with all subjects the DIg^+ cells consistently corresponded to the brightest staining with class II–specific antibodies. These results indicate that while the T/B cell ratio in dolphin PBL may be similar to that observed in other mammals, their T lymphocytes may constitutively express class II molecules.

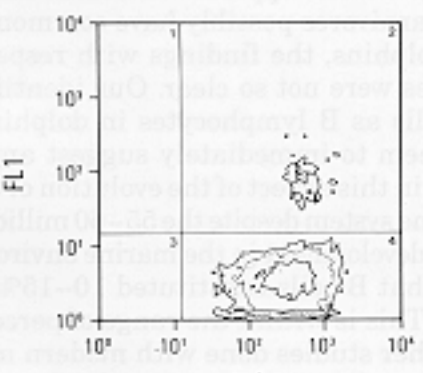

Fig. 8. Contour maps of Tt490M PBL, double-stained with class II–specific Q5/13 Fab2 (FL2) and DIg-specific Fab2 (FL1). Fluorescence is on a log scale.

TABLE 1. Triated thymidine incorporation (cpm) by PBL from Tt490M incubated with indicated concentrations of lectins

Mitogen	Concentration[1]	cpm[2]
Control	—	235 ± 54
ConA[3]	5.0 μg/ml	53,486 ± 8,548
ConA[3]	2.5 μg/ml	63,032 ± 8,013
ConA[3]	1.25 μg/ml	62,647 ± 2,799
Control	—	320 ± 83
PHA[4]	5.0 μg/ml	51,696 ± 2,460
PHA[4]	2.5 μg/ml	60,111 ± 6,857
PHA[4]	1.25 μg/ml	46,209 ± 4,058

[1]Optimized in preliminary experiments.
[2]± standard deviation.
[3]Concanavalin A.
[4]Phytohemagglutinin A.

Several antibodies to T cell markers were tested with dolphin PBL, for the purpose of positive identification. None of the antibodies tried, however, cross-reacted. In order to demonstrate that our dolphin PBL preparation did in fact contain T cells, we tested whether they would proliferate by culturing with the lectins PHA and ConA, typical T cell mitogens in other vertebrate species. Table 1 shows that strong mitogenic stimulation was obtained with both of these lectins, compared to control cultures, suggesting the presence of T cells in the dolphin PBL preparations, probably corresponding to the DIg^-, class II^+ population.

DISCUSSION

Dolphins belong to one of only two orders of mammals to have left the land to adapt to life in a total aquatic environment. It is estimated that they left land 55 to 60 million years ago for the seas (Gingerich et al., '83), evolving in parallel with terrestrial mammals. While it is generally accepted that cetaceans evolved from a primitive ancestor of insectivore stock, there is some debate as to whether cetaceans branched off from the carnivores or ungulates (Slijper, '62). The purpose of this study was to investigate aspects of the cetacean cellular immune system, namely the characteristics and expression of

class II molecules on dolphin PBL, and the percentage of T and B lymphocytes on dolphin PBL, while drawing comparisons with homologous features in terrestrial mammals.

In this study, a monoclonal antibody directed against human class II molecules, Q5/13, was found to be cross-reactive with dolphin class II molecules by several criteria: 1) in immunoprecipitations Q5/13 reacted with molecules that by SDS-PAGE analyses closely resemble the heterodimeric composition of human class II molecules; 2) this structural similarity was confirmed by two-D gel electrophoresis, which also showed the presence of the intracellular subunit Ii, and strongly suggested the possibility that dolphin class II β chains, as in humans, are genetically polymorphic; 3) Q5/13 was reactive by indirect immunofluorescence and flow cytometry with dolphin lymphocytes.

In humans, the region of class II molecules recognized by Q5/13 is located on the β subunit of HLA-DR, -DP, and -DQ molecules and is monomorphic. Q5/13 reacted with 90–99% of dolphin PBL from 21 subjects tested, suggesting that as in humans, the Q5/13 epitope is expressed at the cell surface and is monomorphic in dolphin as well. The three other antibodies against human class II molecules that reacted with only 50–80% PBL may have a lower affinity for dolphin class II molecules than Q5/13 or may only recognize a population of class II molecules that is differentially expressed on lymphocytes.

The fact that Q5/13 reacted with 90–99% of dolphin PBL was surprising since in the best characterized immune systems, those of humans and mice, class II–positive PBL (B lymphocytes and monocytes) are normally in the 10–20% range. To address this issue, we produced antisera reactive with dolphin immunoglobulins, which were capable of immunoprecipitating surface Ig from labeled dolphin PBL. By immunostaining with these DIg-specific antibodies, Ig-bearing B cells accounted for 10–15% of our dolphin PBL preparations. By two-color fluorescence, we were able to distinguish a DIg^+, class II^+ population, accounting for 10–15% of the total and corresponding to B cells, and a DIg^-, class II^+ population accounting for 85–90% of PBL. It is unlikely that this latter population represents monocytes, since these cells were excluded from the FACS analysis by gating based on size and granularity. Furthermore, these PBL preparations were strongly stimulated by well-known T cell mitogens like PHA and ConA. We therefore conclude that this cell population represents peripheral blood dolphin T cells expressing class II molecules.

In the periphery, human T lymphocytes generally do not express class II molecules, but they become class II^+ when activated (e.g., by mitogen stimulation in vitro) (Kaufman et al., '84). Class II expression is therefore considered a marker for T cell activation. It is possible that, in dolphin, T lymphocytes are in a state of continuous activation. Life in the aquatic environment may be a contributing factor to such activation. In this respect, it may be of interest that individual variability in the levels of class II expression was observed. It is unlikely that captivity itself could have influenced this parameter, since we tested eighteen subjects from three distinct environments, and in the apparent absence of infectious pathology. It would be desirable to test blood from subjects in the wild, however, to further clarify this point.

Alternatively, expression of class II on peripheral T cells may reflect physiological species differences. A few studies have reported an unusually high expression of class II molecules on T lymphocytes in some land mammals. Thistlethwaite et al. ('83) were the first to demonstrate readily detectable class II expression on swine PBL, while Lunney and Pescovitz ('87) reported that $CD8^+$ lymphocytes in swine express class II antigens. High levels of class II antigen expression have been demonstrated in unstimulated PBL of the domestic cat (Neefjes et al., '86). Deeg et al. ('82) reported that most mature canine T lymphocytes express class II–like molecules. These studies suggest a similarity in the immune system of dolphin and some carnivores (dog and cat) and ungulates (swine), rather than similarities with the human immune system. This is reasonable, since dolphins are thought to have been evolutionarily related to the carnivores or ungulates.

While our findings on the expression of class II molecules give some support to both ideas that ungulates and carnivores possibly have common ancestry with dolphins, the findings with respect to B lymphocytes were not so clear. Our identification of DIg^+ cells as B lymphocytes in dolphin blood does not seem to immediately suggest any great divergence in this aspect of the evolution of the dolphin immune system despite the 55–60 million years of separate development in the marine environment. We found that B cells constituted 10–15% of dolphin PBL. This is within the range of percentages found in other studies done with modern methods similar to ours (e.g., in humans and rodents).

In this study, lymphocytes were also identified by morphological criteria (i.e., size and granularity) among leukocytes isolated by gradient centrif-

ugation procedures originally developed in the human system. While we made efforts to optimize such procedures for dolphin, some intrinsic limitations are obvious. While the bulk of the lymphocytes was consistently evaluated in our FACS analyses, several other cell populations were deliberately gated out to simplify analyses, or might have been missed. For instance, in some cases, a minor population of large, non-granular, DIg^+, class II^+ cells, presumably B cells, was observed. Obviously, more work is needed to understand fully lymphocyte subsets in dolphin. For future studies, it will be desirable to obtain immunologic reagents capable of directly identifying dolphin T cells.

Hopefully, these investigations will help to provide a better understanding of the properties of the mammalian immune system, by uncovering aspects of its evolutionary adaptation.

ACKNOWLEDGMENTS

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